

Inventors: Lockridge and Watkins
Serial No.: 09/478,739
Filed: December 26, 2000
Page 2

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application.

LISTING OF CLAIMS

1. (Currently Amended) A butyrylcholinesterase variant comprising substantially the same amino acid sequence shown as SEQ ID NO: 2, wherein said variant or said functional fragment thereof has a Tryptophane at amino acid position 328.

2. (Currently Amended) The butyrylcholinesterase variant or functional fragment of claim 1, having a 15-fold increase in cocaine hydrolysis activity, ~~or functional fragment thereof.~~

3. (Withdrawn) A nucleic acid encoding a butyrylcholinesterase variant comprising substantially the same nucleic acid sequence shown as SEQ ID NO: 1, or fragment thereof.

4. (Withdrawn) A butyrylcholinesterase variant comprising substantially the same amino acid sequence shown as SEQ ID NO: 4, or functional fragment thereof.

Inventors: Lockridge and Watkins
Serial No.: 09/478,739
Filed: December 26, 2000
Page 3

5. (Withdrawn) The butyrylcholinesterase variant of claim 4, having a four-fold increase in cocaine hydrolysis activity, or functional fragment thereof.

6. (Withdrawn) A nucleic acid encoding a butyrylcholinesterase variant comprising substantially the same nucleic acid sequence shown as SEQ ID NO: 3, or fragment thereof.

7. (Withdrawn) A butyrylcholinesterase variant comprising substantially the same amino acid sequence shown as SEQ ID NO: 6, or functional fragment thereof.

31
8. (Withdrawn) The butyrylcholinesterase variant of claim 7, having a four-fold increase in cocaine hydrolysis activity, or functional fragment thereof.

9. (Withdrawn) A nucleic acid encoding a butyrylcholinesterase variant comprising substantially the same nucleic acid sequence shown as SEQ ID NO: 5, or fragment thereof.

10. (Withdrawn) A butyrylcholinesterase variant comprising substantially the same amino acid sequence shown as SEQ ID NO: 8, or functional fragment thereof.

11. (Withdrawn) The butyrylcholinesterase variant of claim 10, having a three-fold increase in cocaine hydrolysis activity, or functional fragment thereof.

Inventors: Lockridge and Watkins
Serial No.: 09/478,739
Filed: December 26, 2000
Page 4

12. (Withdrawn) A nucleic acid encoding a butyrylcholinesterase variant comprising substantially the same nucleic acid sequence shown as SEQ ID NO: 7, or fragment thereof.

13. (Withdrawn) A library comprising butyrylcholinesterase variants having at least one amino acid alteration in one or more regions of butyrylcholinesterase corresponding to amino acid positions 68-82 (SEQ ID NO: 9), 110-121 (SEQ ID NO: 10), 194-201 (SEQ ID NO: 11), 224-234 (SEQ ID NO: 12), 277-289 (SEQ ID NO: 13), 327-332 (SEQ ID NO: 14) or 429-442 (SEQ ID NO: 15) of butyrylcholinesterase or functional fragment thereof, said library having at least one butyrylcholinesterase variant exhibiting enhanced cocaine hydrolysis activity compared to butyrylcholinesterase, with the proviso that a butyrylcholinesterase variant having a single amino acid alteration is not the human butyrylcholinesterase having Y at position 328.

14. (Withdrawn) The library of claim 13, wherein said butyrylcholinesterase variants have at least two amino acid alterations.

15. (Withdrawn) The library of claim 13, wherein said at least one amino acid alteration is in the region of butyrylcholinesterase corresponding to amino acid positions 68-82 (SEQ ID NO: 9) of butyrylcholinesterase, or functional fragment thereof.

Inventors: Lockridge and Watkins
Serial No.: 09/478,739
Filed: December 26, 2000
Page 5

16. (Withdrawn) The library of claim 13, wherein said at least one amino acid alteration is in the region of butyrylcholinesterase corresponding to amino acid positions 110-121 (SEQ ID NO: 10) of butyrylcholinesterase, or functional fragment thereof.

17. (Withdrawn) The library of claim 13, wherein said at least one amino acid alteration is in the region of butyrylcholinesterase corresponding to amino acid positions 194-201 (SEQ ID NO: 11) of butyrylcholinesterase, or functional fragment thereof.

18. (Withdrawn) The library of claim 13, wherein said at least one amino acid alteration is in the region of butyrylcholinesterase corresponding to amino acid positions 224-234 (SEQ ID NO: 12) of butyrylcholinesterase, or functional fragment thereof.

19. (Withdrawn) The library of claim 13, wherein said at least one amino acid alteration is in the region of butyrylcholinesterase corresponding to amino acid positions 277-289 (SEQ ID NO: 13) of butyrylcholinesterase, or functional fragment thereof.

20. (Withdrawn) The library of claim 13, wherein said at least one amino acid alteration is in the region of butyrylcholinesterase corresponding to amino acid positions 327-332 (SEQ ID NO: 14) of butyrylcholinesterase, or functional fragment thereof.

Inventors: Lockridge and Watkins
Serial No.: 09/478,739
Filed: December 26, 2000
Page 6

21. (Withdrawn) The library of claim 13, wherein said at least one amino acid alteration is in the region of butyrylcholinesterase corresponding to amino acid positions 429-442 (SEQ ID NO: 15) of butyrylcholinesterase, or functional fragment thereof.

22. (Withdrawn) A library comprising nucleic acids encoding butyrylcholinesterase variants, each nucleic acid having at least one codon encoding at least one amino acid alteration in one or more regions of butyrylcholinesterase corresponding to amino acid positions 68-82 (SEQ ID NO: 9), 110-121 (SEQ ID NO: 10), 194-201 (SEQ ID NO: 11), 224-234 (SEQ ID NO: 12), 277-289 (SEQ ID NO: 13), 327-332 (SEQ ID NO: 14) or 429-442 (SEQ ID NO: 15) of butyrylcholinesterase, or functional fragment thereof, at least one of said nucleic acids encoding a butyrylcholinesterase variant having enhanced cocaine hydrolysis activity compared to butyrylcholinesterase, with the proviso that a butyrylcholinesterase variant having a single amino acid alteration is not the human butyrylcholinesterase having Y at position 328.

23. (Withdrawn) The library of claim 22, wherein said at least one amino acid alteration encoded by said at least one codon is in the region of butyrylcholinesterase corresponding to amino acids 68-82 (SEQ ID NO: 9) of butyrylcholinesterase, or functional fragment thereof.

Inventors: Lockridge and Watkins
Serial No.: 09/478,739
Filed: December 26, 2000
Page 7

24. (Withdrawn) The library of claim 22, wherein said at least one amino acid alteration encoded by said at least one codon is in the region of butyrylcholinesterase corresponding to amino acids 110-121 (SEQ ID NO: 10) of butyrylcholinesterase, or functional fragment thereof.

25. (Withdrawn) The library of claim 22, wherein said at least one amino acid alteration encoded by said at least one codon is in the region of butyrylcholinesterase corresponding to amino acids 194-201 (SEQ ID NO: 11) of butyrylcholinesterase, or functional fragment thereof.

B1
26. (Withdrawn) The library of claim 22, wherein said at least one amino acid alteration encoded by said at least one codon is in the region of butyrylcholinesterase corresponding to amino acids 224-234 (SEQ ID NO: 12) of butyrylcholinesterase, or functional fragment thereof.

27. (Withdrawn) The library of claim 22, wherein said at least one amino acid alteration encoded by said at least one codon is in the region of butyrylcholinesterase corresponding to amino acids 277-289 (SEQ ID NO: 13) of butyrylcholinesterase, or functional fragment thereof.

28. (Withdrawn) The library of claim 22, wherein said at least one amino acid alteration encoded by said at least one codon is in the region of butyrylcholinesterase corresponding to amino acids 327-332 (SEQ ID NO: 14) of butyrylcholinesterase, or functional fragment thereof.

Inventors: Lockridge and Watkins
Serial No.: 09/478,739
Filed: December 26, 2000
Page 8

29. (Withdrawn) The library of claim 22, wherein said at least one amino acid alteration encoded by said at least one codon is in the region of butyrylcholinesterase corresponding to amino acids 429-442 (SEQ ID NO: 15) of butyrylcholinesterase, or functional fragment thereof.

30. (Withdrawn) A method of hydrolyzing a cocaine-based butyrylcholinesterase substrate comprising contacting said butyrylcholinesterase substrate with a butyrylcholinesterase variant shown as SEQ ID NO: 2, or functional fragment thereof, under conditions that allow hydrolysis of cocaine into metabolites, wherein said butyrylcholinesterase variant exhibits a five-fold or more increase in cocaine hydrolysis activity compared to butyrylcholinesterase.

31. (Withdrawn) A method of treating a cocaine-induced condition comprising administering to an individual an effective amount of a butyrylcholinesterase variant shown as SEQ ID NO: 2, or functional fragment thereof, exhibiting increased cocaine hydrolysis activity compared to butyrylcholinesterase.

32. (Withdrawn) The method of claim 31, wherein said cocaine-based substance is cocaine.

33. (Withdrawn) The method of claim 32, wherein said individual is symptomatic of a cocaine-overdose.

34. (Withdrawn) The method of claim 32, wherein said individual is symptomatic of cocaine addiction.

Inventors: Lockridge and Watkins
Serial No.: 09/478,739
Filed: December 26, 2000
Page 9

35. (Withdrawn) A method of hydrolyzing a cocaine-based butyrylcholinesterase substrate comprising contacting said butyrylcholinesterase substrate with a butyrylcholinesterase variant selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, or functional fragment thereof, under conditions that allow hydrolysis of cocaine into metabolites, wherein said butyrylcholinesterase variant exhibits a two-fold or more increase in cocaine hydrolysis activity compared to butyrylcholinesterase.

36. (Withdrawn) A method of treating a cocaine-induced condition comprising administering to an individual an effective amount of a butyrylcholinesterase variant selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, or functional fragment thereof, exhibiting increased cocaine hydrolysis activity compared to butyrylcholinesterase.

37. (Withdrawn) The method of claim 36, wherein said cocaine-based substance is cocaine.

38. (Withdrawn) The method of claim 37, wherein said individual is symptomatic of a cocaine-overdose.

39. (Withdrawn) The method of claim 37, wherein said individual is symptomatic of cocaine addiction.